Concordance of Pathophysiological Responses in Mice Exposed to Different Biomass Smoke Conditions via Aspiration and Inhalation

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Abstract & Background

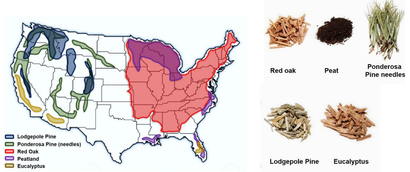
We have previously reported that aspiration of an equal mass (100 µg) of particulate matter (PM) in flaming biomass smoke condensate caused greater lung toxicity in mice than samples from smoldering smoke. In this study, we conducted inhalation exposures on a subset of the biomass smoke fuels and conditions, and compared with the previous results before and after dosimetric adjustment for inhaled PM. Biomass smoke from peat, eucalyptus and oak fuels was generated under smoldering and flaming phases with PM levels precisely maintained by an automated smoke emission controlling system. Mice were exposed for 1 hour/day for 2 days and then assessed for lung toxicity at 4 and 24 h after the second exposure. PM levels were ~40 and ~4 mg/m³ from the smoldering and flaming phases, respectively, while carbon monoxide (CO) levels ranged between ~60 to 110 ppm depending on the fuel and combustion conditions. Total inhaled PM in the mouse lungs during the exposure was estimated to be ~130 and ~13 µg PM, for smoldering and flaming respectively. Peat smoke produced under either combustion conditions caused similar increases in neutrophil (PMN) influx at both time points despite the flaming PM concentration being 10-fold lower. PMN responses to smoldering eucalyptus were higher than flaming at 4 h although effects were equivalent for both conditions by 24 h. A significant increase in ventilator timing (as measured by Penh), potentially indicating airflow obstruction, was observed in mice exposed to flaming peat and for both flaming and smoldering eucalyptus immediately after each day of exposure, in agreement with the inflammation results. No pathophysiological responses were seen following exposure to either combustion condition of oak, which mirrored the responses following aspiration exposure. Overall the results show good concordance in responses between aspiration and inhalation studies depending on type of fuel and combustion conditions and confirm that PM from flaming condition is, on a mass basis, more toxic than that from smoldering smoke.
[This abstract does not represent EPA policy]

Research background

- Wildland fire smoke is a hazardous mixture of gaseous emissions and particulate matter (PM).
- Inhalation exposure is the gold-standard approach for studying inhaled agents, but is difficult to apply to biomass smoke PM due to considerable variability and less reproducibility of PM concentrations within inhalation studies.
- Well control of PM concentrations is a key factor in the design of biomass smoke inhalation study.

Materials & Methods

Tested biomass fuels and their distribution in the United States

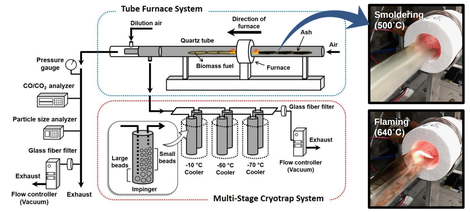


- Red oak (obtained from the Air Pollution Prevention and Control Division at the US EPA)
- Peat (collected from the coastal plain of the eastern North Carolina, ARNWR)
- Ponderosa pine needles (provided by the Missoula Fire Sciences Laboratory)
- Lodgepole pine (provided by the Missoula Fire Sciences Laboratory)
- Eucalyptus (purchased from a local supplier)

Materials & Methods

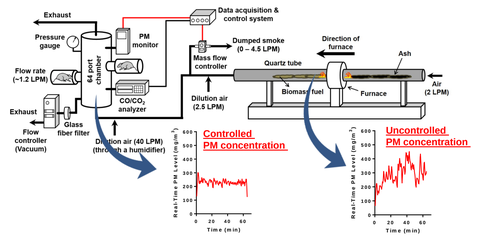
Aspiration Exposure Study

Biomass combustion and smoke collection system

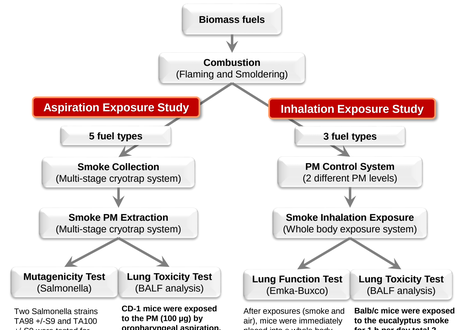


Inhalation Exposure Study

Automated combustion and smoke inhalation system



Flow diagram of the biomass smoke study



Two Salmonella strains TA98 +/-S9 and TA100 +/-S9 were tested for mutagenicity assay.

CD-1 mice were exposed to the PM (100 µg) by oropharyngeal aspiration. BALF was analyzed at 4 h and 24 h post exposure.

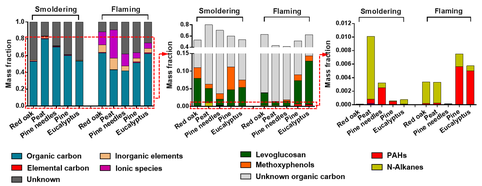
After exposures (smoke and air), mice were immediately placed into a whole body plethysmograph system to measure lung function parameters.

Balb/c mice were exposed to the eucalyptus smoke for 1 h per day total 2 days. BALF was analyzed at 4 h and 24 h post exposure.

Results

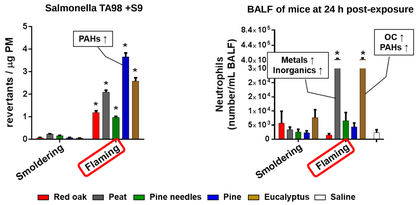
Aspiration Exposure Study

Figure 1: Chemical mass fractions of the biomass smoke PM (an equal mass basis)



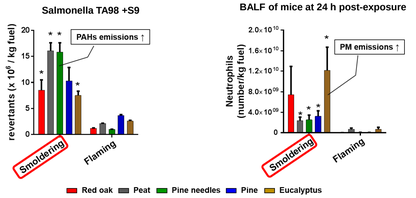
- Levels of organic carbon and levoglucosan were dependent on fuel types (woody vs. non-woody fuel).
- Levels of ions, inorganic elements, and methoxyphenols were dependent on combustion phases (smoldering vs. flaming).

Figure 2: Mutagenic and lung toxicity potencies of the PM (an equal mass basis)



- Mutagenicity and lung toxicity of the PM were greater in the flaming phase smoke than the smoldering smoke on an equal PM mass basis.

Figure 3: Mutagenicity and lung toxicity EFs of the PM (an emission factor basis)

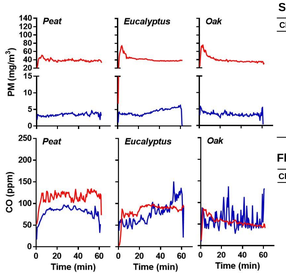


- Mutagenicity and lung toxicity of the PM were greater in the smoldering phase smoke than the flaming smoke on an emission factor (EF) basis.

Results

Inhalation Exposure Study

Figure 4: Biomass smoke properties in the inhalation chamber



Smoldering combustion condition

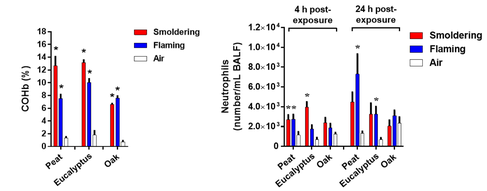
Characteristic	Peat	Eucalyptus	Oak
PM (mg/m³)	38.7±0.4	42.2±0.6	40.5±0.8
CO (ppm)	115±1	84±2	56±1
CO_2 (ppm)	721±5	427±8	297±5
NO (ppb)	227±54	30±1	39±0
NO_2 (ppb)	0±0	0±0	0±0
NOx (ppb)	227±54	30±1	39±0
VOCs (ppb)	2,677	1,911	4,072
MMD (nm)	151	283	180

Flaming combustion condition

Characteristic	Peat	Eucalyptus	Oak
PM (mg/m³)	3.4±0.1	4.2±0.1	3.5±0.1
CO (ppm)	81±1	76±2	59±2
CO_2 (ppm)	2,794±40	4,790±78	2,942±65
NO (ppb)	5,109±892	1,449±61	1,812±233
NO_2 (ppb)	1,292±187	666±19	502±154
NOx (ppb)	6,380±1,079	2,106±77	2,303±384
VOCs (ppb)	1,694	760	1,682
MMD (nm)	133	165	158

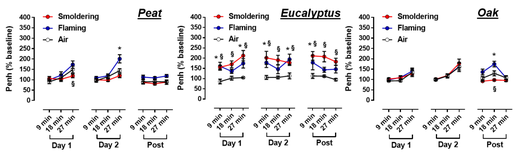
- Smoldering PM levels were ~10 times higher than flaming PM with CO held at similar levels to equalize potentially interfering CO health effects.

Figure 5: Biological responses to the biomass smoke



- Smoldering peat and eucalyptus smoke elicited significant inflammation (neutrophils) at 4 h post-exposure while flaming smoke from either fuel caused even greater lung inflammation at 24 h post-exposure.

Figure 6: Lung function responses to the biomass smoke



- A significant increase in ventilatory timing (as measured by Penh) was observed in mice exposed to flaming peat (*) and eucalyptus (*), and smoldering eucalyptus (§) smoke immediately after each day of exposure, in agreement with the inflammation results.

Results

Figure 7: Lung toxicity: Aspiration vs. Inhalation

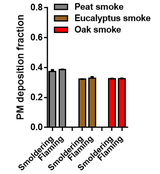
Administered PM dose

			Peat	Eucalyptus	Oak
Aspiration dose (µg)	4 h	Smoldering	100	100	100
		Flaming	100	100	100
	24 h	Smoldering	100	100	100
		Flaming	100	100	100
Inhalation dose (µg)*	4 h	Smoldering	66	61	97
		Flaming	6	5	9
	24 h	Smoldering	66	61	97
		Flaming	6	5	9

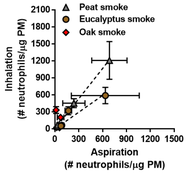
*Inhalation dose = PM concentration x deposition fraction x total respiratory volume (minute volume x 120 min)

The deposition fraction was determined by multiple-path particle dosimetry (MPPD) model.

PM deposition fraction in entire respiratory tract



Lung toxicity potency Aspiration vs. Inhalation



- Concordance of lung toxicity potencies between the inhalation and aspiration methods was observed in mice exposed to the peat (p<0.0001) and eucalyptus (p=0.0056) smoke but not the oak (p=0.3270) smoke.

Conclusions

- Type of fuel and combustion conditions have dramatic differences in emission characteristics, mutagenicity, and lung toxicity.
- Two different ways of expressing toxicological outcomes (based on a potency and emission factor) should be considered in assessing the health effects of wildland fires.
- Inhalation studies conducted with the automated combustion system can validate responses seen in aspiration exposure studies after adjustment for PM dosimetry.
- Wildland fire smoke in the rich regions of peat and eucalyptus fuels may induce greater health effects than smoke from oak fires.
- The automated combustion system is capable of controlling combustion phases and PM concentrations and also can be employed for health risk assessment from inhalation exposure to wildfire smoke.

Future Work

Photochemically aged biomass smoke study